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Nitrate and fecal coliform concentration differences at the soil/bedrock interface in Appalachian silvopasture, pasture, and forest

Douglas G. Boyer · James P. S. Neel

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Abstract A major limitation to efficient forage-based livestock production in Appalachia is asynchrony of forage availability and quality with nutritional requirements of the grazer. Producers require dependable plant resources and management practices that improve the seasonal distribution and persistence of high quality herbage, sustainability and environmental integrity of the agricultural landscape. It was hypothesized that inorganic N and fecal coliform concentrations delivered in leachate to the soil/bedrock interface would be lowest in deciduous forest (HF) and highest in pasture (CP) with HF converted to silvopasture (SP) between the two. Piezometers were used to monitor water quality at the soil/bedrock interface under conventional pasture, SP, and hardwood forest. The pasture and SP were rotationally grazed by sheep during the spring to fall grazing season (2004–2008). Geometric mean fecal coliform bacteria concentrations (FC) were greatest in SP (18 FC 100 mL^{-1}) with no difference between CP (7.5 FC 100 mL^{-1}) and HF (5.6 FC 100 mL^{-1}). Mean NO_3-N concentration was lowest in SP (2.3 mg L^{-1}) and greatest in CP (4.4 mg L^{-1}) and HF (4.1 mg L^{-1}), which were not significantly different. Mean NH_4-N concentrations showed different trends with the lowest mean concentration in CP (0.5 mg L^{-1}) and the greatest in SP (2.5 mg L^{-1}) and HF (2.6 mg L^{-1}), which were not significantly different. SP was shown to be a management option in the study area that reduces nitrate leaching, but should be considered cautiously in near-stream areas and near wells where fecal bacteria pollution can be problematic. This study makes an important contribution to our knowledge about interactions between landscape management and environmental quality of the Appalachian region. A diversity of land and forage management options are needed to maximize forage and livestock productivity while protecting surface and groundwater quality of the region.

Keywords Nitrate · Fecal coliforms · Subsurface water · Macropores

D. G. Boyer (✉) · J. P. S. Neel
US Department of Agriculture, Appalachian Farming Systems Research Center, Agricultural Research Service, 1224 Airport Rd., Beaver, WV 25813, USA
e-mail: doug.boyer@ars.usda.gov

Introduction

Efficient forage-based livestock production in Appalachia is limited by asynchrony of forage availability and quality with nutritional requirements of the grazer. Producers require dependable plant resources and management practices that improve the seasonal distribution and persistence of high quality herbage, sustainability, and environmental integrity of agricultural landscapes. Silvopastoral agroforestry systems are being investigated for potential production and environmental benefits on topographically complex landscapes common to the Appalachian region (Feldhake and Schumann 2005).

The Appalachian region in the eastern United States covers an area of nearly 531,000 km^2. As a headwaters area, the Appalachian region produces about 3.4×10^{11} m^3 of water annually flowing to the major eastern population centers of the United States, the bays and estuaries on the Atlantic coast, and to the Gulf of Mexico. Thousands of small farm families and small rural communities of the region depend on clean water for health, industry, and livelihood. Protection of the Appalachian region's water resources has local, regional, and national implications. Agroforestry practices on the region's diverse landscapes might be beneficial for protection of water quality.

High infiltration capacities of undisturbed soils (Anderson et al. 1976), macropores (Holden 2008), thin soils and fractured bedrock (Levison and Novakowski 2009) are common to Appalachian hillslopes and contribute to rapid recharge of shallow groundwater (Kipp and Dinger 1987). Shallow conduit flow groundwater systems found in karst terrain are also common in Appalachia and respond quickly to agricultural activities on the surface (Boyer and Pasquarell 1996, 1999; Boyer and Kuczynska 2003).

Deciduous forest sites of the region are characterized by thick surficial organic layers and extensive systems of macropores (Carmean 1957). Manipulation of forest to provide silvopastoral forage production creates an actively changing soil system that transforms from a forest soil toward a pasture soil. The soil properties probably stabilize somewhere between a forest and pasture soil. The rate and magnitude of changes are unknown. Staley et al. (2008) found that conversion of deciduous forest to silvopasture (SP) resulted in soil chemistry changes indicative of rapid transition to improved pasture. Surface litter changes and remnant macropores in SP recently established from deciduous forest resulted in changes in organic carbon transport to the soil/bedrock interface (Boyer and Neel 2007). Grazing land management system effects on nutrient cycling and the fate and transport of pathogens require careful study in order to develop environmentally effective systems that protect public health.

Numerous field and model studies have shown the linkage between hillslope hydrology and N-cycling and fecal coliform bacteria (FC) transport (e.g., Cirmo and McDonnell 1997; Dorner et al. 2006). The objective of the study was to confirm or refute our hypotheses that mean inorganic N (NO_2-N, NO_3-N, and NH_4-N) and fecal coliform concentrations delivered in leachate to the soil/bedrock interface would be lowest in hardwood forest (HF), highest in pasture (CP), and mean concentrations in HF converted to SP would fall between the two, or $C_{HF} < C_{SP} < C_{CP}$, where C = mean concentration.

Materials and methods

The study site is located in southern West Virginia at 37°47′39″N, 80°58′22″W at 884 m elevation (Fig. 1).

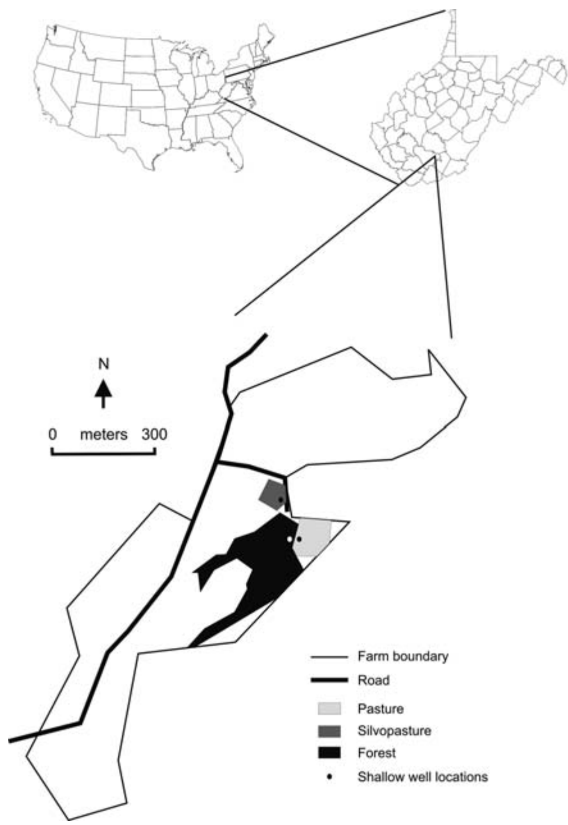

Fig. 1 Shallow well locations in pasture, SP, and forage sites at the Appalachian Farming Systems Research Center farm in Raleigh County, West Virginia, USA (37°47′39″N, 80°58′22″W, 884 m elevation). The shallow wells are placed about 10 m apart along transects in each of the land uses. Inset maps show West Virginia location in the USA (*top left*) and farm and Raleigh County location in West Virginia (*top right*)

The research farm, which is owned and operated by the Appalachian Farming Systems Research Center, is composed primarily of a mosaic of pasture and mixed hardwood forest. The conventional pasture is composed primarily of orchard grass (*Dactylis glomerata* L.), Kentucky bluegrass (*Poa pratensis* L.), and white clover (*Trifolium repens* L.) and has been maintained as pasture for many years. The CP is surrounded by mixed HF, part of which was converted to SP in 2001 by thinning to allow ~50% full sunlight and planting Benchmark orchard grass, BG34 perennial ryegrass (*Lolium perenne* L.), and Huia white clover. The area was subsequently thinned to allow 70–80% full sunlight in fall 2003 to ensure forage stand sustainability. The trees in the SP and HF are primarily white oaks (*Quercus* alba L.) with minor amounts of sugar maple (*Acer saccharum* L.), and yellow poplar (*Liriodendron tulipifera* L.). The areas of the CP and SP paddocks used in this study were each about 0.1 ha. A similar sized area of the HF was used for study. The CP and SP areas were rotationally grazed by sheep during the growing season (April–October). The rotational grazing schedule was dependent on availability of forage. Sheep were rotated onto the SP and CP paddocks for about a week every 35 days. The SP and CP sites were maintained as low-input systems and primarily received surface-applied, granular, inorganic fertilizer as dictated by soil test results in the earlier establishment years prior to establishment of rotational grazing. N supplied by the fertilizers was derived from ammonium nitrate in the 34–0–0 fertilizer and a combination of urea, ammonium sulfate, ammonium nitrate and ammonium phosphate in the mixed NPK fertilizers. SP received 33.6 kg N ha^{-1} (34–0–0) in April 1999, 33.6 kg N ha^{-1} (19–19–19) in April 2000, 33.6 kg N ha^{-1} (19–19–19) in May 2002, and 33.6 kg N ha^{-1} (34–0–0) in July 2003. CP received 22.4 kg N ha^{-1} (10–20–20) in May 1999, 33.6 kg N ha^{-1} (19–19–19) in August 2001, and 33.6 kg N ha^{-1} (34–0–0) in July 2003. Other soil amendments in SP included 6.7 MT ha^{-1} dolomitic lime in February 1999 and May 2004, 6.5 MT ha^{-1} dolomitic lime in April 2001, 112 kg ha^{-1} P_2O_5 (0–46–0) in April 1999, 90 kg ha^{-1} P_2O_5 (0–46–0) in April 2001, and 874 kg ha^{-1} of 0-25-25 fertilizer in May 2005. Additional soil amendments to CP were 4.5 MT ha^{-1} dolomitic lime in May 1999 and 857 kg ha^{-1} of 0–25–25 fertilizer in March 2005. No soil amendments were made on the HF.

The soils in the research area were formed from acid materials weathered from siltstones, shales, and sandstones of the New River and Pocahontas Formations of the Pennsylvanian Pottsville Group (Cardwell et al. 1968). The research sites were located on a Gilpin (fine-loamy, mixed, semiactive, mesic Typic Hapludult) and Lily (fine-loamy, siliceous, mesic Typic Hapludult) soil association.

A 30 years mean annual precipitation at the nearby (13 km west) Raleigh County Memorial Airport in Beaver, WV is 106 cm, of which 62% falls during the April–October growing season. Mean annual air temperature is 10.9°C with mean monthly temperatures ranging from a low of −0.9°C in January to 21.5°C in July. Average annual snowfall is 154 cm.

Four 1.9 cm diameter drive-point piezometers (Solinst, Georgetown, Ontario, Canada) were installed with a slide hammer to refusal at the soil/bedrock interface in each land use category (CP, SP, and HF). Piezometers were installed midway between the nearest two trees in the SP and HF sites. Piezometers were installed in a line diagonally across the paddocks resulting in the piezometers being about 10 m apart. The lowest 30 cm of each piezometer was stainless steel and screened. Variable depths to bedrock resulted in the piezometers being installed to average depths of 145 (102, 246, 169, 65), 204 (229, 192, 208, 187), and 145 (128, 221, 117, 114) cm in the CP, SP, and HF sites, respectively. Water samples were retrieved from the piezometers with a peristaltic pump (Geotech Environmental Equipment, Inc., Denver, CO) and 4.8 mm diameter silicone tubing shortly after storms. Samples were collected during the end of storms or within a few hours of storm end since a temporary, shallow, perched water table was being sampled. The water table was usually gone in less than 24 h following storms. The perched water table rarely appeared during extended dry periods. Water samples were transported on ice back to the laboratory in sterile plastic bottles. The water samples were quickly filtered through 0.45 μm membrane filters which were placed on mFC nutrient agar media in Petri plates. FC were counted on the filter media following incubation at 44.5°C for 22–24 h and recorded as colony forming units (CFU) per 100 mL. The sample water that passed through the filters was analyzed by suppressed ion chromatography (Dionex Corp., Sunnyvale, CA) for inorganic nitrogen (NO_2, NO_3 and NH_4) and recorded as mg N L^{-1}. Since

NO_2 usually converts quickly to NO_3, the two ions were combined in this study and are simply referred to as NO_3-N.

Samples were collected from September 2004 through December 2008. Sample results were grouped for seasonal analyses by categorizing samples as spring (March–May), summer (June–August), fall (September–November), and winter (December–February). Sheep rotationally grazed the SP and CP sites from April to October. FC counts were normalized by adding one to all FC counts and transforming to logarithms (base 10) for all statistical analyses and calculation of geometric means. Statistical tests were done with the Statistical Analysis System (2008). Piezometers were treated as replicates within each land use. Differing perched water table responses to rainfall resulted in unequal numbers of samples so the GLM procedure with the repeated measures option was used to test for land use, season, and landuse*season interaction effects on mean concentrations. When significant differences were indicated a least significant means in a Tukey–Kramer multiple range comparison test was used to test for differences between individual land uses. All statistical tests are significant at the $P \geq 95\%$ level unless stated otherwise.

Results

Water appeared at the soil/bedrock interface more frequently in SP than the CP or HF sites. The total number of storm samples collected at the SP, CP, and HF sites was 321, 196, and 73, respectively, but not all samples were analyzed for all parameters for various analytical and logistical reasons. The temporary, perched water table appeared most frequently in SP and least frequently in HP accounting for the major difference in numbers of samples collected. Occasionally sufficient sample volumes were not great enough for FC testing, which had a target of 30–60 countable colonies per filter plate. Table 1 shows the summary statistics for inorganic N and FC concentrations in each of the land use treatments. Mean inorganic N (NO_3-N + NH_4-N) did not differ between land uses. Separating the inorganic N ions into NO_3-N and NH_4-N showed that mean NO_3-N concentration in SP was about half the concentration in CP and HF. Mean NH_4-N concentration in SP and HF was about five times the mean concentration in CP. Geometric mean fecal coliform concentration in SP was two to three times greater than the geometric mean concentration in CP and HF.

The GLM test results for land use, season, and landuse*season interaction effects on mean NO_3-N, NH_4-N, and log FC concentrations are shown in Tables 2, 3, and 4, respectively. Significant land use differences as indicated by least significant means in a Tukey–Kramer multiple range test are indicated in Table 1. The GLM results showed highly significant treatment, season, and landuse*season interaction effects on mean concentrations in all but the landuse*season interaction effect on mean NO_3-N concentrations.

Seasonal mean NO_3-N concentrations are shown in Fig. 2a. Mean NO_3-N concentrations in the SP leachates are lower than the concentrations in CP or HF leachates in all seasons, except summer. Mean

Table 1 Summary statistics for inorganic N and fecal coliform concentrations in silvopasture (SP), pasture (CP), and hardwood forest (HF)

	SP		CP		HF	
	Mean ± SE[1,2]	n[3]	Mean ± SE	n	Mean ± SE	n
Inorganic N (mg L^{-1})	4.9 ± 0.61[a]	298	5.0 ± 0.35[a]	188	6.3 ± 1.48[a]	70
NO_3-N	2.3 ± 0.28[a]	309	4.4 ± 0.32[b]	196	4.1 ± 0.76[b]	73
NH_4-N	2.5 ± 0.47[a]	321	0.5 ± 0.10[b]	193	2.6 ± 1.32[a]	72
Fecal coliforms (CFU 100 mL^{-1})	18.0 ± 1.21[a]	294	7.5 ± 1.28[b]	167	5.6 ± 1.45[b]	69

Means with the same letter are not significantly different within rows

1 Geometric mean (mean of the logarithms transformed back to a real number) for fecal coliforms

2 Standard error for fecal coliforms is the anti-log of the standard error of log concentration

3 Number of samples

Table 2 Analysis of variance (GLM) results for NO_3-N versus treatment (land use), season, and treatment*season

Source	Degrees of freedom	Mean sum of squares	Significance (%)
Model error	566	24.4	
Landuse	2	289.4	>99
Season	3	173.4	>99
Landuse*season	6	23.2	54

Table 3 Analysis of variance (GLM) results for NH_4-N versus treatment (land use), season, and treatment*season

Source	Degrees of freedom	Mean sum of squares	Significance (%)
Model error	574	48.4	
Landuse	2	256.2	99
Season	3	1,073.8	>99
Landuse*season	6	209.2	>99

Table 4 Analysis of variance (GLM) results for log fecal coliforms versus treatment (land use), season, and treatment* season

Source	Degrees of freedom	Mean sum of squares	Significance (%)
Model error	518	1.44	
Landuse	2	9.89	>99
Season	3	91.59	>99
Landuse*season	6	5.06	>99

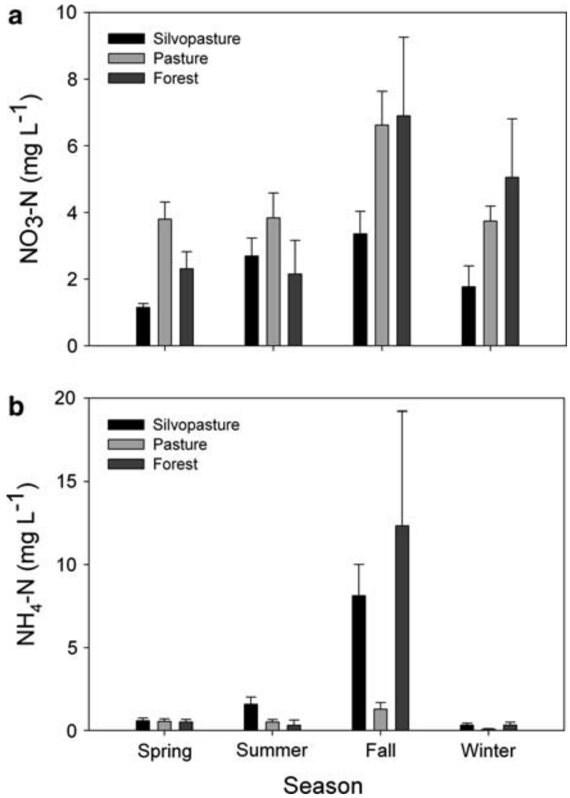

Fig. 2 Mean seasonal **a** NO_3-N and **b** NH_4-N concentrations at the soil/bedrock interface in SP, pasture, and HF land uses. Spring = Mar–May, Summer = June–Aug., Fall = Sept.–Nov., Winter = Dec.–Feb. *Error bars* represent standard error

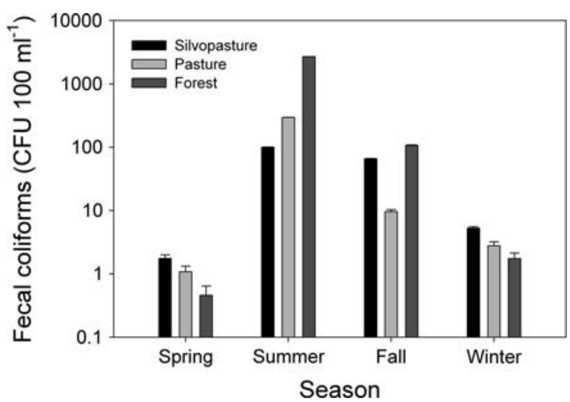

Fig. 3 Geometric mean seasonal FC concentrations at the soil/bedrock interface in SP, pasture, and HF land uses. Spring = - Mar–May, Summer = June–Aug., Fall = Sept.–Nov., Winter = Dec.–Feb. *Error bars* represent standard error

NO_3-N concentrations were greatest in fall with CP and HF NO_3-N concentrations averaging 6.6 and 6.9 mg L^{-1}, respectively. The fall NO_3-N concentration in SP averaged 3.4 mg L^{-1}. Seasonal mean NH_4-N concentrations (Fig. 2b) were much greater in fall in SP (8.12 mg L^{-1}) and HF (12.33 mg L^{-1}) than in CP (1.28 mg L^{-1}) or in any other season.

Geometric mean FC concentrations at the soil/bedrock interface were highest in summer in all three land use treatments (Fig. 3) and next highest in fall. FC concentrations gradually decreased through winter to their lowest concentrations in spring. The highest geometric mean FC concentrations during the growing season occurred in the forest site, which was not grazed by sheep. Trend analysis using linear regression failed to show any significant trend in log FC concentrations in the CP or HF land uses, but a highly significant ($P \geq 99.9\%$) decreasing trend

(0.23 log CFU 100 mL^{-1} $year^{-1}$) in the SP land use. The significant trend in SP was primarily a result of high FC counts in the first year of study. When the first year of data is eliminated from analyses there is no significant FC trend, but the geometric mean FC concentration is still highest in SP.

Cumulative frequency analyses of the occurrence of NO_3-N concentrations show similar results for each of the land uses up to about 30% of the samples (see Fig. 4a). At the 30% level NO_3-N concentrations increase more rapidly in CP and least rapidly in SP. The CP and HF cumulative frequency curves converge at about 80% of the samples and the SP samples remain lower until about 95% of the samples where all three curves are similar. Higher FC concentrations are shown for SP in Fig. 4b. A 40% of the samples from SP had FC concentrations $\leq$1 CFU 100 mL^{-1}. More than 60% of the CP and HF samples had FC concentrations $\leq$1 CFU 100 mL^{-1}.

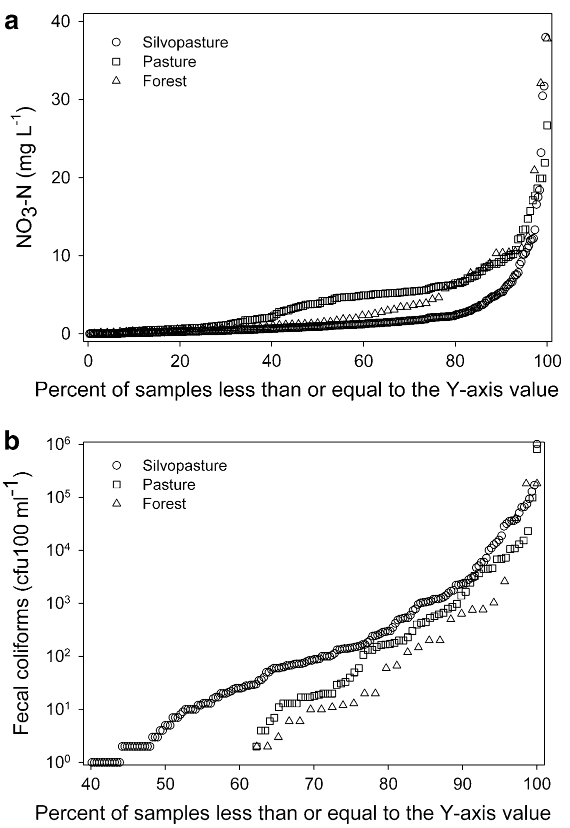

Fig. 4 Cumulative distributions of **a** NO_3-N and **b** FC concentrations at the soil/bedrock interface in SP, pasture, and HF

Discussion

The low NO_3-N concentrations at the soil/bedrock interface in SP relative to those in CP and HF suggests SP can be a management option from a water quality standpoint. NO_3-N concentration in SP was hypothesized to be greater than the concentration in HF and lower than that in CP. The forage plants in SP were probably using much of the NO_3-N that would otherwise leach to the tree root zone. Other studies have found that trees are able to capture much of the NO_3-N that leaches past the herbaceous root systems in SP and agroforestry buffers (Huxley et al. 1994; Schroth 1995; Udawatta et al. 2002; Dougherty et al. 2009). Staley et al. (2008) found that soil chemical characteristics of these same SP sites were rapidly approaching pasture-like conditions, but they did not study NO_3-N. Over time, SP NO_3-N might be expected to approach the higher concentrations found in CP because of fertilizer amendments needed for forage growth, but regression analysis of NO_3-N concentration versus time failed to show any movement in that direction. The higher fall NO_3-N concentrations in CP and HF are probably related to return of N with leaf fall (Muller and Martin 1983) in HF and senescence of white clover (Whitehead 1995) in CP (see Fig. 2a).

Organic nitrogen originating from plant materials, manure, and urine are mineralized to inorganic nitrogen primarily by ammonification into NH_4^+. The mineralization process is primarily carried out by heterotrophic microorganisms that prefer aerobic conditions. The NH_4^+ is then nitrified by nitrifying bacteria into NO_2^- and then quickly to NO_3^{-2}, which is the form of inorganic N that is readily leached. Figure 2b shows the greatly increased concentrations of NH_4-N in the transient shallow groundwater of the SP and HF sites during the fall. Fall leaf drop probably supplied the plant material that resulted in seasonally high levels of NH_4-N. The HF site had a mean NH_4-N concentration about 1.5 times greater than the SP site in the fall. The mean NO_3-N concentration in HF was twice the mean NO_3-N concentration in SP during the fall. Since the SP site was created by thinning hardwood forest to allow ~79–80% full sunlight less leaves were available for fall leaf drop. The CP site contained no trees and seasonal mean NH_4-N concentration was about 10% of the HF NH_4-N concentration. Mean fall NO_3-N concentrations in CP were greatly elevated and may have resulted from senescing of white clover roots. Similar results for elevated fall NO_3-N concentration in root zone water were found by Boyer and Belesky (unpublished data) in comparisons of orchard grass versus orchard grass/white clover mix in an earlier study on pasture.

The high geometric mean FC concentrations in SP were unexpected and might have resulted from a well-organized system of macropores and disturbance of the surface litter layer. Dougherty et al. (2009) found lower *E. coli* concentrations in tile drain effluent from a mixed tree intercrop than in tile drain effluent from a corn monocrop. Removal of some of the organic litter layer in SP diminishes the site's ability to intercept water before it reaches mineral soil, and the macropores provide a fairly direct route to deeper soil layers bypassing much of the filtration provided by the soil matrix. Dougherty et al. (2009) suggested that microbiological activity associated with tree roots might have a detrimental effect on *E. coli* survival. Although *E. coli* concentrations were not reported in this study, a large number of samples in the latter part of this study were analyzed for *E. coli* as well as FC, and *E. coli* concentrations were typically accounted for 95–100% of the measured FC concentrations. Gagliardi and Karns (2002) found that plant roots enhanced persistence of *E. coli* O157:H7 and microbial activity, but they did not study tree roots. Macropores might have been providing a bypass mechanism for FC bacteria to avoid roots. Higher macroporosities have been found in agroforestry buffer strips compared to grass buffer strips (Udawatta et al. 2006). Boyer et al. (2009) found that macropores readily transmitted *Cryptosporidium* oocysts, which are two to four times greater in size than FC, through undisturbed soil columns. The high FC concentrations at the soil/bedrock interface in HF were not expected since livestock were not grazed in the vicinity of the piezometers. Results shown in Fig. 4b indicate that FC were not transported to the soil/bedrock interface on a regular basis (more than 60% of the HF samples did not test positive for FC). The intermittent high FC counts in HF water samples could have resulted from wild animal activity. Meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*), raccoons (*Procyon lotor*), and white-tailed deer (*Odocoileus virginianus*) are commonly seen in the area and all are known sources of FC. Pathogenic bacteria are known to colonize the rhizosphere (Berg et al. 2005), but *Escherichia coli*, the primary species making up FC, usually dies off to a small population (Stoddard et al. 1998). Most, if not all, water samples would be expected to contain FC if the bacteria were naturally surviving in the rhizosphere. Wild animals cannot be excluded as sources of FC in the SP samples, but a much higher percentage of samples in SP tested positive for FC indicating that sheep manure was a source of bacteria.

Regression analysis indicates that geometric mean FC concentrations are decreasing over time, but there is no trend in CP or HW. The regression coefficient shows that geometric mean FC concentrations in SP are expected to be similar to the geometric mean FC concentration in CP after 5–6 years if the trend continues. However, the SP sites should retain some of their macropore features and geometric mean FC concentrations could remain greater than those in pasture at the soil/bedrock interface. FC bacteria reaching the soil/bedrock interface are expected to experience a tortuous route through bedrock fractures, often in-filled with sediment, and never reach surface water. A higher portion of flow in CP might be overland flow providing fecal FC a more direct route to surface water.

Results from this study show that NO_3-N contributions to shallow groundwater are less under SP than conventional pasture. Macropores in SP systems created by thinning hardwood forest stands seem to enhance transport of fecal organisms to shallow groundwater, but the tortuous flow route through fractured bedrock to surface water might minimize impacts on surface water quality. Study of the effects of tree and herbaceous plant roots on FC is needed to better understand FC transport through soil in SP systems created from thinned hardwood forest. Ungrazed buffers along surface streams might provide effective barriers to fecal bacterial contamination from SP areas where overland flow is minimized. SP was shown to be a management option in the study area that reduces nitrate leaching, but should be considered cautiously in near-stream areas and near wells where fecal bacteria pollution can be problematic. This study makes an important contribution to our knowledge about interactions between landscape management and environmental quality of the Appalachian region. A diversity of land and forage management options are needed to maximize forage and livestock productivity while protecting surface and groundwater quality of the region.

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